

and how important it is to take this into account when planning vaccine or intervention strategies. Several intriguing questions remain regarding pneumococcal carriage, transmission, and evolutionary adaptation. Are carriage strains of *S. pneumoniae* in SCD patients continuously reseeded from strains carried by the GP, or are these transmitted from SCD patient to patient? If the latter is true, this would mean that the pneumococcus is undergoing parallel but separate linear evolutionary pathways in these

two populations. The use of “designer” vaccines to target these populations should be considered.

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Endosomes as Platforms for NOD-like Receptor Signaling

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<http://dx.doi.org/10.1016/j.chom.2014.05.001>

The NOD-like receptors (NLRs) were among the first innate immune receptors discovered, yet a clear understanding of the basic principles underlying their mechanisms of action is lacking. Two recent studies provide important cell biological insights into the subcellular sites of NOD1- and NOD2-dependent signaling.

The modern field of innate immunity is centered on the study of several families of pattern recognition receptors (PRRs). These receptor families include the Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs), RIG-I-like receptors (RLRs), and AIM2-like receptors (ALRs). Each of these receptor families functions to detect microbial products in a multicellular host and induces one or more antimicrobial responses that prevent infection. As such, much work in this area has focused on understanding how PRRs detect microbial products and the mechanisms of subsequent signal transduction. Despite being one of the first PRR families discovered (Philpott et al., 2014), insight into the means by which NLRs detect their microbial products lags far behind the other families. Two recent studies have addressed these deficiencies in our knowledge (Irving et al., 2014; Nakamura et al., 2014), providing us with a much clearer understanding of the earliest events asso-

ciated with the activation of NLR-dependent innate immune responses.

NLRs are a structurally related family of proteins, with individual family members serving distinct biological functions (Philpott et al., 2014). For example, some NLRs act as transcription factors in the nucleus to promote the expression of major histocompatibility complex (MHC) genes, and others act as central regulators of inflammasome activation (Davis et al., 2011). The assignment of NLRs as PRRs came from studies of the NOD1 and NOD2 proteins, which were found to activate NF- κ B-dependent cytokine expression during infections with bacteria that enter the cytosol. Reductionist studies subsequently revealed that NOD1 and NOD2 can be activated by specific components of the bacterial cell wall, such as d-glutamyl-meso-diaminopimelic acid (iE-DAP) in the case of NOD1 and muramyl dipeptide (MDP) in the case of NOD2 (Philpott et al., 2014). Despite this knowledge, several confusing aspects of NOD1 and

NOD2 biology remained. For example, it has been difficult to detect direct interactions between these receptors and their proposed ligands, whereas similar inquiries into receptor-ligand interactions with other PRR families have been successful. This issue has been further complicated by the recent discovery that some NLR family members do not bind to microbial products directly, but rather interact with upstream proteins of the NAIP family that bind to specific microbial ligands (Kofoed and Vance, 2011). These findings raised the possibility that at least some NLR family members are not actually PRRs, but are adaptor proteins that facilitate the signaling functions of upstream PRRs. A second confusing aspect of NLR biology has emerged from studies demonstrating that natural NOD ligands can trigger NF- κ B activation when added to the extracellular media of cultured cells (Kaparakis et al., 2010). This finding was surprising, since the NODs are cytosolic proteins. Thus, it has long been suspected

that a transporter in the endolysosomal network of mammalian cells facilitates the delivery of NOD ligands to cytosolic receptors. The nature of this proposed transporter has remained elusive, as is the site in the cell where NOD-dependent signaling actually occurs.

Two independent studies by Mellman and colleagues and Kaparakis-Liaskos and colleagues have addressed these deficiencies in our knowledge (Irving et al., 2014; Nakamura et al., 2014) and in the process identified endosomal membranes as sites of NOD1/NOD2-dependent signal transduction. The study by Mellman sought to address the means by which the NOD2 ligand MDP is released from endosomes in dendritic cells (DCs). Using a proteomic approach to query phagosomes, the authors

searched for transporters that may mediate the movement of small peptides across membranes. SLC15A3 and the related protein SLC15A4 were the most promising candidates. The authors found these proteins to be highly enriched on endosomal membranes, and prior work demonstrated their ability to transport peptides across membranes using the proton gradient as an energy source (Daniel and Kottra, 2004; Lee et al., 2009). This latter point was interesting, as MDP-induced NF- κ B activation is sensitive to chemicals that disrupt the acidic nature of endosomes (Lee et al., 2009). Several elegant experiments implicated SLC15A3 in the transport of MDP and the activation of NOD2. For example, DCs lacking SLC15A3 exhibited substantial defects in cytokine expression induced by MDP-coated beads or bacteria, whereas cellular responses to LPS were unaffected. In addition, studies in 293T epithelial cells ruled out a role for these transporters in the innate immune responses to flagellin and TNF. Perhaps most convincingly, the authors cleverly engineered a mutant of SLC15A3 that is localized to the plasma membrane, rather than endosomes. As compared to cells expressing endosomal SLC15A3, cells

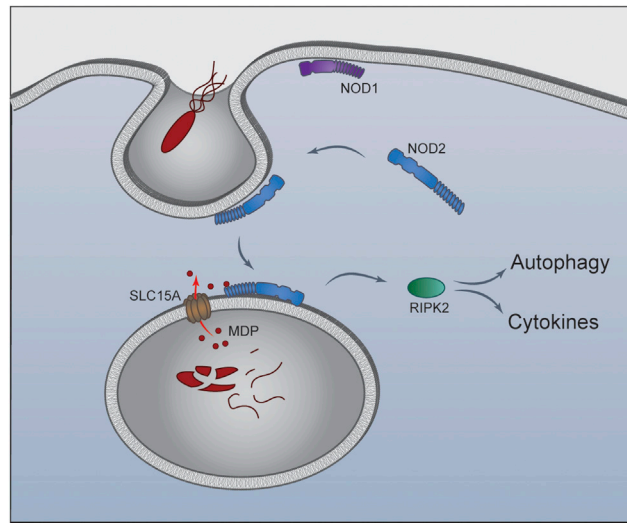


Figure 1. Proposed Model to Explain the Initiation of NOD1- and NOD2-Dependent Signal Transduction

Host mammalian cells can internalize the bacteria they encounter by phagocytosis. NOD proteins may be recruited to sites of phagocytosis by an unknown mechanism. The NODs then remain attached to the formed phagosome and will detect microbial cell wall components after they are released in the degradative endosomal network. The peptide transporter SLC15A3 likely functions to transport NOD ligands across endosomal membranes to the receptor(s), which then initiates innate immune signal transduction.

expressing plasma membrane-localized SLC15A3 exhibited an enhanced ability to deliver extracellular MDP to the cytosol and an enhanced ability to induce cytokine expression. The enhancement of signaling likely results from a bypass of the endocytosis bottleneck that usually limits the amount of MDP that can be delivered to endosomes containing this transporter. Thus, using both loss- and gain-of-function approaches, Mellman and colleagues convincingly implicate SLC15A3 in the transport of MDP to NOD2. These data, coupled with prior work suggesting a role of SLC15A4 in NOD-dependent signaling (Lee et al., 2009), ensure that research into SLC15A3 and SLC15A4 biology will continue to drive the field for some time.

A final intriguing observation made from this study provides a link to the work of Kaparakis-Liaskos. Both groups sought to identify the subcellular site of NOD-dependent signal transduction, and both arrived at endosomal membranes as the likely site of these important events. Using bacterial outer membrane vesicles (OMVs) as a natural source of NOD ligands (Karakakis et al., 2010), Kaparakis-Liaskos and colleagues found that OMV-containing endosomes recruit NOD1 and the

downstream kinase RIPK2. This recruitment correlated with NOD-dependent cytokine secretion and the induction of autophagy. Evidence was also presented to indicate direct interactions between NOD1 and bacterial OMVs. These experiments were important as they provide the first direct evidence of an interaction between NOD1 and a natural (not chemical) ligand. Similarly, the Mellman group used MDP-containing beads (or *Salmonella*) to demonstrate the recruitment of NOD1, NOD2, and RIPK2 to endosomal membranes. Interestingly, phagosomes containing NOD ligands also displayed SLC15A3, which further supports its role in transporting microbial products.

The fact that NOD1 and NOD2 signaling proceeds from endosomal membranes is consistent with prior work

indicating that these proteins are not simply cytosolic receptors that diffuse through the cell in search of their ligands (Barnich et al., 2005; Travassos et al., 2010). Rather, ample evidence now supports the idea that NLRs (at least in the case of NOD1 and NOD2) are peripheral membrane proteins that survey one or more organelles for bacterial cell wall components. A notable distinction between these current works and the earlier studies is the prior assignment of NOD1 and NOD2 as proteins localized to the cell surface. Indeed, early studies from Podolsky and colleagues identified NOD2 at the surface of epithelia and found a NOD2 variant that is functionally implicated in the pathology of Crohn's disease to be cytosolic (Barnich et al., 2005). Mellman's work also examined the localization of this Crohn's variant of NOD2 and found it to be mislocalized to the cytosol. A possible explanation for the differences in NOD localization observed by different groups is that NOD proteins may have the ability to bind different organelles. We propose that NOD proteins may first be recruited from the cytosol to the cell surface during the process of endocytosis or phagocytosis (Figure 1). This recruited pool of NODs

may then “ride” into the cell on the nascent vesicles and await the release of bacterial cell wall fragments by a process facilitated by SLC15A3 (Figure 1). If correct, then understanding the mechanisms of NOD recruitment to the cell surface or endosomes may be an interconnected question, and NODs may have a single domain within their sequence that permits interactions with proteins (or lipids) in both locations of the cell. In this regard, it is worth noting recent work demonstrating that the TLR adaptor protein TIRAP functions in this way (Bonham et al., 2014), interacting with lipids at the cell surface and endosomes to facilitate the detection of active receptors. Once detection of active TLRs is achieved, TIRAP recruits its downstream partner MyD88 to initiate signal transduction (Bonham et al., 2014). It will be important to understand when NODs are first recruited to membranes. Are these proteins targeted to membranes immediately after they are synthesized (like TIRAP), or are they recruited by a

signal-dependent process (like MyD88)? Follow-up research into this new exciting area of NOD cell biology will surely provide answers to these questions and may help explain the operation of the other receptor families of the innate immune system.

ACKNOWLEDGMENTS

J.C.K. is supported by NIH grants AI093589 and AI072955 and an unrestricted gift from Mead Johnson & Company. J.C.K. holds an Investigators in the Pathogenesis of Infectious Disease Award from the Burroughs Wellcome Fund. K.S.B. is supported by a predoctoral fellowship from NSF.

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